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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
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Identification of a novel liquid phosphate phosphatase prg-1 (plasticity related
gene-1) involved in axonal outgrowth and regenerative sprouting

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Identification of a novel liquid phosphate phosphate ^{AIC}PRG-1 (plasticity related gene-1)
involved in axonal outgrowth and regenerative sprouting

Description

Axons in the central nervous system (CNS) elongate through the extracellular space over long distances (1). This occurs during development (2, 3) and during axonal sprouting in response to partial deafferentation (4, 5). The extracellular space, however, is an outgrowth repellent environment that allows axonal elongation only under specific molecular conditions (6). Molecules involved in axonal outgrowth, such as semaphorins, netrins, or ephrins (7, 8, 9, 10), are able to transduce outgrowth promoting as well as inhibiting signals to elongating axons via specific receptors. Here, we provide evidence for a novel molecular mechanism by which axons are able to elongate in a phospholipid-rich environment that would normally inhibit outgrowth of fibers (11, 12). Specific upregulation of this novel member of the lipid phosphate phosphatase (LPP) family acting as an ecto-enzyme on axons allows local depletion of lipid phosphates, enabling fiber outgrowth through the repellent extracellular environment.

In the hippocampus, afferent connections from the entorhinal cortex enter in a layer-specific manner during development (13). This specific axonal navigation depends on molecular cues expressed along the pathway and in the target region (13). Transection of entorhinal axons in the adult leads to a specific deafferentation in the hippocampus with subsequent regenerative axon sprouting by remaining afferents into the denervated zones (4, 14). In an attempt to identify the molecular cues that govern this structural plasticity, we performed a differential cDNA screening approach for mRNAs specifically upregulated during development and in the lesioned hippocampus (15). We identified a new gene of 716 amino acids (aa) (Fig. 1A) which we named plasticity-related gene-1 (PRG-1; GenBank acc. #, submitted). This gene encodes a yet uncharacterized member of the LPP-1 membrane-associated phosphatic acid phosphatase ecto-enzyme family. These molecules have 6 membrane spanning domains with their active site located on the external surface of the plasma membrane. They have increasingly attracted interest because they are involved in modulating the specific signaling of bioactive lipid phosphates such as phosphatidate (PA), lysophosphatidate (LPA) or

sphingosine-1-phosphate (S-1-P) in the context of cell migration, mitogenesis, and neurite retraction (16, 17, 18). It has been shown that signaling via extracellular LPA plays an important role in CNS development and that postmitotic neurons are at least one endogenous source for LPA in the nervous system (19). Similar to other members of the LPP-family, hydrophobicity analysis of PRG-1 predicts six N-terminal membrane-spanning regions with highly conserved phosphatase domains. However, unlike any other member of this family, the second half of the protein consists of a long hydrophilic domain of around 400 aa (Fig. 1B). According to the structural models of LPP orientation in the membrane, this C-terminal extension is positioned on the cytoplasmatic site and might thus play a role as a regulatory or signal transduction domain. Besides the homology of the N-terminal part of PRG-1 to other members of the LPP-family such as LPP-1 and the Drosophila cell migration modulator Wunen, GenBank searches revealed only one other related gene (genomic DNA sequence: GenBank acc. # NP_079164), which we cloned and named PRG-2 (GenBank acc. #, submitted). This gene shares the same C-terminal extension with partial sequence homology, but shows a different expression pattern than PRG-1 (data not shown). Thus, these genes represent a novel distinct subclass of the LPP-1 family. Amino acid residues which have been shown to be essential for ecto-enzyme activity in the LPP-1 class of proteins are conserved in PRG-1 N-terminal sequences (Fig. 1A) (20, 21). Database analysis of the C-terminal domains did not detect any significant similarities to any other protein or any matches with known conserved domains (ProDom and Swiss-Prot databases). GenBank search for orthologous proteins show that both genes are highly conserved in mammals (human/mouse > 93%), and partial EST sequences indicate orthologous proteins in Xenopus and Zebrafish, whereas no significant homology for the C-terminal part could be found in the Drosophila or other invertebrate genome. Northern blot analysis revealed one distinct band and shows that PRG-1 mRNA expression is CNS-specific, with the exception of a weak expression in testis (Fig.

1C). Thus, PRG-1 appears to be a novel vertebrate specific protein, selectively located in the brain with putative phosphatase function.

In situ hybridization analysis highlighted tight regulation of PRG-1 transcripts in the developing hippocampus (15, 22). At embryonic day 16 (E16), no PRG-1 transcripts can be detected in the brain (Fig. 1D). An expression signal first appears at E19 in the subventricular zone and specifically in the hippocampal anlage, whereas other cortical regions do not show PRG-1 expression (Fig. 1D). From postnatal stages on, PRG-1 mRNA is present in the hippocampus and in the entorhinal cortex throughout adult stages (Fig. 1D). In the dentate gyrus, a region bearing postnatally developing granule cells (23), weak PRG-1 mRNA expression is found in the infrapyramidal blade at P0, whereas the later developing suprapyramidal blade first showed expression signals at P5. This expression pattern remains unchanged during maturation; however, a reduced expression is apparent in the adult brain.

We next analyzed PRG-1 mRNA expression following entorhinal cortex lesion which leads to a layer-specific denervation of the hippocampus followed by regenerative ingrowth of sprouting axons (5, 14, 27). PRG-1 is upregulated one day after lesion (dal) and peaks at 5 dal in the ipsilateral hippocampus (gcl = 37%, hilus 300%, CA1 = 100%, CA3 = 60%). The contralateral hippocampus (maximum by 1 dal, gcl = 16%, hilus = 200%, CA1 = 59%, CA3 = 46%), as well as the ipsilateral cortex, shows a strong upregulation of PRG-1 mRNA (maximum by 1 dal 83%) (Fig. 1E).

Transfection studies using a PRG-1 construct tagged with the eGFP reporter gene reveals its processing in COS-7 cells through the Golgi apparatus (data not shown) to its final localization in the plasma membrane of small processes (Fig. 2A). To localize PRG-1 protein *in vivo*, we generated an antiserum against a peptide sequence from the cytoplasmic C-terminus of PRG-1 (15). This antiserum specifically stains transfected COS-7 cells expressing PRG-1-eGFP fusion protein (Fig. 2A). Both Western blot and immunostaining could be blocked by specific peptide incubation prior to the antiserum (data not shown).

Immunocytochemical staining of rat hippocampus reveals PRG-1 specifically in neurons (Fig. 2B) (15). Five days after entorhinal cortex lesion, a clear immunoreactive PRG-1 positive band appears in the denervated outer molecular layer (Fig. 2B), apparently representing single axonal processes which form terminal branches (Fig. 2B). To show that PRG-1 is indeed localized in regrowing axons following lesion, we performed an ultrastructural analysis. Immunoreactivity could indeed be localized to growth cone-like axonal structures in the denervated zones of the hippocampus (Fig. 2C).

Members of the LPP-family are known to dephosphorylate extracellular phospholipids such as lysophosphatidic acid (LPA). To study the phosphatase activity of PRG-1, we chose LPA as a simple phospholipid that has properties of an extracellular neurite repellent factor (16, 29). It is present in the extracellular space of the central nervous system (19, 30) and mediates diverse cellular responses through the activation of multiple signal transduction pathways (17, 24). One major structural effect of LPA on neurons is rapid neurite retraction with subsequent cell rounding. In order to provide evidence that PRG-1 is involved in axonal outgrowth during hippocampal development, we studied the effect of PRG-1 expression on LPA induced neurite retraction in living brain tissue. *In vivo*, axons start to grow from the entorhinal cortex towards the hippocampus at about E19 (3), which is parallel to the developmental onset of PRG-1 expression (Fig. 1D). Thus, we compared the response of entorhinal explants obtained before PRG-1 expression (at E16) to that of postnatal explants which express PRG-1 (15). Both embryonic and postnatal explants grow equally well under serum-free culture conditions and show long extending axons. However, their response to LPA differed dramatically (Fig. 3A). Whereas application of 10 μ M LPA leads to rapid neurite retraction in embryonic entorhinal explants (E16; n = 32), postnatal explants (P0; n = 36) did not differ significantly from vehicle treated control cultures (Fig. 3B). These experiments indicate that postnatal entorhinal axons expressing PRG-1 are resistant to LPA-induced neurite retraction. To test if differential PRG-1 expression is directly responsible for these effects, we expressed PRG-1

protein in neuronal cell lines known to show a rapid retraction of their processes in response to LPA. N1E-115 cells are uniformly sensitive to LPA-induced growth cone collapse (Fig. 4). Expression of PRG-1 in these cells renders the axonal growth cones resistant to LPA-induced collapse (Fig. 4; 15). Transfection with a control vector solely containing the reporter gene does not alter the LPA-induced responses (Fig. 4). Moreover, analysis of stress fiber formation by phalloidin staining revealed prevention of LPA-induced actin-polymerization (11) in PRG-1 overexpressing cells when compared to controls (data not shown; 15). These data directly demonstrate that PRG-1 expression interferes with LPA-mediated signaling, thus preventing neurite retraction (Fig. 4B).

Sequence alignment analysis revealed that the catalytic domains of lipophosphate phosphatase activity of the LPP family are conserved in PRG-1. These domains catalyze the conversion of phosphatidic acid to diacylglycerol and inorganic phosphate, and are postulated to function both in lipid biosynthesis and in cellular signal transduction (25, 26). In order to confirm a phosphatase action of PRG-1, we exchanged the conserved catalytic histidine (His-252) with lysine by site specific mutagenesis (15). This exchange has been shown to completely abolish enzymatic function of the catalytic center of LPP-1 (18). The same exchange abolished germ cell guiding activity of the *Drosophila* Wunen protein (21). Transfection of this construct into N1E-115 cells no longer prevented LPA-induced retraction of processes as achieved by the wt-construct. These findings provide evidence for the fact that the conserved enzymatic domain present in the LPP-1 family is necessary for attenuating LPA-induced neurite retraction (18).

In this study, we describe the identification and functional characterization of a novel lipid phosphate phosphatase, PRG-1, that is specifically expressed in neurons. PRG-1 is upregulated during hippocampal development only after E19 and following ECL, both periods characterized by active axonal outgrowth. Since embryonic neurons, at a time point before their outgrowth from the entorhinal cortex, lack PRG-1 expression, it appears to be the

onset of PRG-1 expression on entorhinal axons that allows them to elongate just after birth and thus penetrate a LPA-rich environment. Moreover, upregulation of PRG-1 expression in regrowing axons following ECL occurs at a post-lesion time point when fibers from the reinnervating associational/commissural tract commence to invade into the denervated entorhinal termination zone (16, 17, 29). In addition, the subcellular localization to axons and growth cone-like structures strengthens the concept that PRG-1 plays an important role in axon growth both during development and regenerative sprouting.

LPA is present in the extracellular space (19, 30) and is known to act via lysophosphatidate receptors (EDGs), involving intracellular activation of small G-proteins that mediate neurite retraction (24, 28, 29). Our *in vitro* studies suggest that PRG-1 antagonizes the activation of EDGs, presumably by reducing the local concentration of active phospholipids surrounding axonal growth cones (see schematic diagram). In fact, a mutant PRG-1 protein lacking a critical residue in the active site was no longer able to protect from LPA-induced neurite retraction. This indicates that PRG-1 expression is a prerequisite for developmental axon growth and regenerative sprouting. This way, PRG-1 is able to regulate activation of lysophosphatidate receptors (EDGs) and thereby modulate axonal outgrowth. Therefore, our data provide evidence for a novel mechanism of axonal outgrowth through the phospholipid-rich environment during development and following brain injury.

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31. The authors thank Denis Lajko, Peggy Thiele, Miriam Petzold, Elke Bürger and Gisela Duwe for expert technical support; Sabine Lewandowski and Dore Wachenschwanz for expert help with photography and graphic design; Trina Irico for editorial assistance; and Drs. Gregory Wulczyn and Frauke Zipp for critical remarks on the manuscript. This study was supported by the DFG: SFB 515/A5.

Figure legends:

Figure 1:

PRG-1 mRNA expression is brain-specific.

A. Human PRG-1 amino acid sequence (GenBank acc. #, submitted). The first 300 aa are highly conserved to LPP family members. The other 400 aa (blue boxed sequence) of PRG-1 show no homologies to known sequences. The catalytic histidin (His-252) conserved in all members of the LPP-superfamily is marked with an asterisk. We exchanged this amino acid with lysine to study the functional consequences. B. Hydrophobicity profile of the PRG-1 protein predicted by the Kyte and Doolittle algorithms. Numbers in the bottom refer to amino acid residues from the amino terminus. Blue boxed area of PRG-1 is predicted as hydrophilic and located in the cytosol. C. Northern blot analysis of PRG-1 mRNA shows a single 5.5 kb band in brain and in testis. D. Expression pattern of PRG-1 mRNA in the developing and lesioned rat brain. At embryonic day 16 (E16), no hybridization signal can be detected (right side). Toluidine blue staining of the same section is shown on the left side. On E19, a strong hybridization signal is detectable in the subventricular zone and specifically in the CA1-CA3 principal layer of the hippocampus. Toluidine blue staining of this section is shown on the left side. From the day of birth to adulthood, PRG-1 mRNA is present in all principal layers of the hippocampus and entorhinal cortex. After entorhinal cortex lesion, PRG-1 mRNA is significantly upregulated in all areas of the ipsilateral hippocampus (1 - 5 dal), in the contralateral hippocampus (1 dal) and in the ipsilateral cortex (1 - 5 dal). Statistical differences from respective controls are marked with an asterisk (mean \pm S.D.; $n = 6$), ** $P < 0.001$; two-tailed t test with Bonferroni correction for multiple comparisons. (E.) hi = hippocampus; LV = lateral ventricle; LP = lateral posterior thalamic nucleus; LD = laterodorsal thalamic nucleus; btp = basal telencephalic plate, posterior part; CA1 = cornu ammonis; DG = dentate gyrus; RSG = retrosplenial granular cortex; dal = days after lesion;

Scale bar in E19 equals 850 μm and also applies to E16. Scale bar in P30 equals 740 μm and also applies to P0-P15. Scale bar in 1 dal equals 500 μm and also applies to adult.

Figure 2:

PRG-1 is expressed in cellular processes and in hippocampal neurons.

A. Overexpression of PRG-1-eGFP fusion protein (green) in COS-7 show localization in cellular processes. The same subcellular expression pattern can be detected with the anti PRG-1 peptide antibody (red). Colocalization of PRG-1-eGFP and anti PRG-1 shows overlapping in the transfected COS-7 cells and in their processes (marked with white arrows). Scale bar, 10 μm . **B.** Immunocytochemical analysis of PRG-1 in the adult rat hippocampus and after lesion. Pyramidal neurons are labeled in the CA1 and CA3 region. Polymorphic cells are stained in the hilus. Granule cells of the dentate gyrus are also immunopositive. Five days after lesion (dal), a specific increase of immunoreactivity is apparent as an immunopositive band in the outer molecular layer (oml; marked with black arrows). Higher magnification from the boxed area shows immunostained axons (marked with black arrows) and their terminal branches (white arrows). gcl = granule cell layer, hi = hilus. Scale bar equals 580 μm and also applies to adult. Scale bar in 5 dal oml equals 20 μm . **C.** Electron micrograph of a PRG-1 immunopositive axon (delineated by black arrows) with its terminal branch (delineated by red arrows) 5 dal in the oml. Afferent elements including spines are devoid of PRG-1 immunostaining. ax = axon, s = spine. Scale bar equals 0.4 μm .

Figure 3:

Developing entorhinal axons are differentially affected by LPA.

A. Images of representative explants from the rat entorhinal cortex at embryonic day 16 (E 16) and postnatal day 0 (P0) (outgrowing axons marked with white arrows) used to analyze the effect of LPA on neurite retraction. Explants of entorhinal cortex at E16 lack PRG-1

expression, while explants at P0 show high PRG-1 expression levels. The explants were treated with 10 μ M LPA or vehicle (0.9% NaCl) for 10 min. Scale bar equals 20 μ m. B. A scoring system with three different degrees of neurite extensions was used to assess the LPA effect (1: no extensions; 2: short processes; 3: long processes). Experiments were analyzed by three independent observers in a blinded fashion. E16 explants ($n = 20$) show significant retraction after treatment with LPA in contrast to postnatal explants ($n = 22$). Statistical differences from controls are marked with an asterisk (mean \pm S.D.; explants from three independent set of experiments in total: E16, $n = 36$; P0, $n = 38$), * $P < 0.05$; two-tailed t test with Bonferroni correction for multiple comparisons).

Figure 4:

PRG-1 protects from LPA-induced retraction

A. Cell rounding and neurite retraction in response to LPA in N1E-115 cells (wild type = wt). N1E-115 cells were transfected with PRG-1-eGFP fusion construct. For controls, the pEGFP-N1 vector was used. The enzymatic domain of PRG-1 was altered by exchange of the catalytic histidine (aa 252) by lysine (PRG-1^{His/Lys}). Controls transfected with pEGFP-N1 show retraction of neurites and cell rounding after exposure to 10 μ M LPA. PRG-1-eGFP transfected cells treated with 10 μ M LPA for 10 min show no retraction of processes (marked with white arrows). Transfection with the same vector bearing the His-Lys exchange in the catalytic histidine (PRG-1^{His/Lys}) no longer attenuated the LPA induced retraction. Panels on the left show transfected cells, panels in the middle show nuclear staining (Hoechst staining, Roche, Germany), and panels on the right show merged images with f-actin staining. Scale bar equals 20 μ m. **B.** A scoring system with three different levels was used to assess neurite outgrowth (1: cell rounding; 2: short processes; 3: long processes). The results from three independent set of experiments (one set with $n = 40$ for each group) were evaluated by three independent observers in a blinded fashion. Statistical differences from controls are marked

with an asterisk (mean \pm S.D.), *P < 0.05; two-tailed t test with Bonferroni correction for multiple comparisons).

Schematic diagram of the proposed axon growth mechanisms in a phospholipid-enriched environment. (Fig. 5)

Axons that are sensitive to a repulsive phospholipid but do not express PRG-1 are unable to cross a phospholipid-rich barrier. In contrast, PRG-1 expressing neurons can grow through a phospholipid-rich zone by locally depleting the extracellular pool of repulsive phospholipids acting as ligands on EDG receptors. This way, PRG-1 may regulate the activation of EDG receptors and thereby modulate axonal outgrowth.

Supporting Online Material

Material and Methods

Animals and surgery

All animals were housed under standard laboratory conditions, and the surgical procedures were performed in agreement with the German law (in congruence with 86/609/EEC) for the use of laboratory animals. All efforts were made to minimize the number of animals used, and all surgical procedures were performed under sufficient anesthesia to minimize animal suffering.

The experimental procedures are described in detail in Bräuer et al., 2001 (S1).

Suppressed Substraction hybridization

We used the SMART cDNA technology from Clontech to generate high yields of full-length, double-stranded cDNA from adult, control and lesioned hippocampus rat RNA. To develop the subtraction library, we used the Clontech PCR-Select cDNA Substraction Kit. We used the Clontech PCR-Select differential Screening Kit to analyze the differential expressed cDNAs

***In situ* hybridization and quantification**

For hybridization, we used an antisense oligonucleotide (5'- GCA GAG GTC TGA ATT CTA GTG TCT ATC GTT ATA GTT CCT TAA CAG TGT GGG -3') complementary to bases 425 - 475 of a rat EST cDNA clone (GenBank acc. AW 526088.1). The oligonucleotide was synthesized by Metabion (Munich, Germany). The specificity was confirmed by a BLAST GenBank search to rule out cross-hybridization to other genes.

We used the protocol as described by Bräuer et al., 2001 (S1).

Antibody generation and immunohistochemistry

To design a peptide antibody against PRG-1, we used a sequence in the hydrophilic C-terminal region. The peptide (NH₂-CVGVNGDHHVPGNQ-COOH), representing amino acids 490 – 507 of the PRG-1 rat sequence (GenBank acc. # submitted), was synthesized by BioGenes (Berlin, Germany). The amino-terminal cysteinyl residue, which is not part of the PRG-1 sequence, was included for conjugation of the peptide to a carrier protein. The peptide was conjugated through the cysteinyl sulfhydryl to maleimide activation (keyhole limpet hemocyanin). Rabbits were also immunized by BioGenes. The specificity of the peptide antibody against PRG-1 was further tested on Western-Blot and on brain sections by blocking via peptide incubation prior to adding the antiserum. The protocol for the immunohistochemistry is described in detail by Bräuer et al., 2001 (S2).

Subcellular localization

PRG-1 tagged with the eGFP reporter gene was used to identify the subcellular localization. Golgi apparatus was visualized with the cell tracker BODYPY ceramide (Molecular Probes, Netherlands). The staining protocol was obtained from Molecular Probes.

LPA induced neurite retraction in explants

Entorhinal explants from E16 and P0 rat pups were obtained from timed-pregnant Wistar rats and was cultivated as described (S3). In brief, entorhinal cortex were carefully dissected from the hippocampal anlage and the meninges were removed. Explants were gently transferred with a fire-polished Pasteur pipette into 12-well plates and cultivated on baked glass cover slides coated with laminin and poly-L-lysine (25 µg/ml and 10µg/ml, respectively) in culture medium containing selenium-defined fetal bovine serum [5%] (S4) (Neurobasal medium plus 25 µg/ ml Penicillin/Streptomycin; B-27 supplement). After 24 h, culture medium was exchanged and cultivation was further performed in serum-free Neurobasalmedium for 20 h.

Serum-starved explants were treated with oleoyl-LPA for 10 min and then fixed in 4% paraformaldehyde for 20 min. For F-actin staining, fixed tissues were incubated with FITC-phalloidin (0.1 µg/ml, Sigma, Germany) for 40 min, followed by incubation with HOECHST 33258 dye for 5 min at room temperature. After three washing steps in PBS, explants were coverslipped with ImmuMount (Merck, Germany) prior to analysis. For quantification, a scale score system with three degrees of explant axon outgrowth was used: 1 = no extensions; 2 = short processes; 3 = long processes.

Site-directed mutagenesis of PRG-1^{HIS/LYS}

The rat PRG-1 full length clone was amplified by Marathon PCR (Clontech, USA) from adult rat hippocampus RNA (GenBank submitted). For transfection studies, the full length PRG-1 coding sequence was fused to EGFP (pEGFP-N1 vector Clontech, USA). The PRG-1^{HIS/LYS} exchange mutant at the catalytic histidin (His-252) was introduced in the same protein fusion vector by site specific mutagenesis (CAT to AAG).

LPA induced neurite retraction and protection in N1E-115 cells

N1E-115 mouse neuroblastoma cells (ATCC: CRL-2263) were routinely grown in DMEM medium supplemented with selenium-defined fetal bovine serum (10%). The cells were seeded on baked glass cover slides at a density of 10,000 cells/cm². The next day, cells were transfected with the cationic lipid procedure (FuGene6, Roche, Germany) and cultivated for 24 h. Serum-starvation was performed for 20 h in DMEM medium, followed by treatment with 10 µM oleoyl-LPA or vehicle (0.9% sodium chloride) (29). After 10 min, cells were fixed in 4% paraformaldehyde for 20 min at room temperature and analysis was done under an Olympus BX-50 microscope. For quantification, we used a scoring system in accordance with Ebens et al. (1996, S5) with three degrees of explant axon outgrowth: 1 = cell rounding; 2 = short processes; 3 = long processes.

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13. Sep. 2002

10	20		50	60
MQRAGSSGGR	GECDISGAGR	LGLEEAARLS	CAVHTSPGGG	RRPGQAAGMS
70	80	90	100	110
KDSVTLLPCP	YPVELPILAS	SVVSLYFLEL	TDVFKPVHSG	FSCYDRSLSM
130	140	150	160	170
PFLMLLSLAF	AGPAITIMVG	EGILYCCLSK	RRNGVGLEPN	INAGGCNFNS
190	200	210	220	230
VHVFGLCSTA	LITDIIQLST	GYQAPYFLTV	CKPNYTSLSN	SCKENSYIVE
250	260	270	280	290
INSGRKSFPS	QHATLAAPAA	VYVSMYFNST	LTDSSKLLKP	LLVFTFIICG
310	320	330	340	350
YIQHPVDVYC	GFLIGGGIAL	YLGLYAVGNF	LPSDESMFOH	RDALRSLTDL
370	380	390	400	410
KNGSSSDGIA	HTEGILNRNH	RDASSLTNLK	RANADVEIIT	PRSPMGKENM
430	440	450	460	470
NTPSVEDPVR	RNASIHASMD	SARSKQLLTO	WKNKQESRKL	SLQVIEPEPG
490	500	510	520	530
SSSEPSRVGV	NGDHHGPGNQ	YLKIQPGAVP	GCNNSMPGGP	RVSIQSRFGS
550	560	570	580	590
QENISTSPKS	SSARAKWLKA	ABKTVACNRS	NSOPRIMOVI	AMSKQQGVLO
610	620	630	640	650
VSCTGSIRYK	TLTDHEPSGI	VRVEAHPENN	RPIIQIPSTE	GEGSGSWKWK
670	680	690	700	710
YELNDLNRDS	ESCESLKDSF	GSGDRKRSNI	DSNEHHHHGI	TTIRVTPVEG
730	740	750	760	770
SSSRDSTLRR	KGNIILIPER	SNSPENTRNI	FYKGTSPTRA	YKD.....

human PRG 1
SEQ ID NO: 1

10	20	30	40	50	60
MQRAGSSGAR	GECDISGAGR	LRLEQAARLG	GRTVHTSPGG	GLGARQAAGM	SAKERPKGKV
70	80	90	100	110	120
IKDSVTLLPC	FYFVELPILA	SSVVSIFYLE	LTDVFKPVHS	GFSCYDRSL	MPYIEPTQEA
130	140	150	160	170	180
IPFLMLLSLA	FAGPAITIMV	GEGLYCCLS	KRRNGAGLEP	NINAGGCNFM	SFLRRVRFV
190	200	210	220	230	240
GVHVFGLCST	ALITDIIQLS	TGYQAPYFLT	VCKPNYTSN	VSCKENSYIV	EDICSGSDLT
250	260	270	280	290	300
VINSGRKSFP	SQHATLAFA	AVYVSMYFNS	TLTDSSKLLK	PLLVTPIIC	GIICGLTRIT
310	320	330	340	350	360
QYKNHPVDVY	CGFLIGGGIA	LYLGLYAVGN	FLPSEDSMLQ	HRDALRSLTD	LNQDPSRVL
370	380	390	400	410	420
AKNGSSGDGI	AHTEGILMRN	HRDASSLTNL	KRANADVEII	TPRSPMGKES	MVTFSNTLPR
430	440	450	460	470	480
ANTPSVEDPV	RRNASIHASM	DSARSKQLLT	QWKSKNESRK	MSLQVMDTEP	EQSPPPRSIE
490	500	510	520	530	540
MRSSSEPSRV	GVNGDHHVPG	NQYLKIQPGT	VPGCNNSMPG	GPRVSIQSRP	GSSQLVHIPE
550	560	570	580	590	600
ETQENISTSP	KSSSARAKWL	KAARKTVDCN	RSNNQPRIMO	VIAMSKQQGV	LQSSPKNAEG
610	620	630	640	650	660
STVTCTGSIR	YKTLTDHEPS	GIVRVEAHPE	NNRPPIQIPS	STEGBGSGSW	KWKVPEKSSL
670	680	690	700	710	720
RQTYELNDLN	RDSESCESLK	DSFGSGDRKR	SNIDSNEHHH	HGITTIRVTP	VEGSEIGSET
730	740	750	760	770	780
LSVSSSRDST	LRRKGNIIIL	PERSNSPENT	RNIFYKGTSP	TRAYKD

In case PRG-1

SEQ ID NO:2

10	20	30	40	50	60
MISTKEKNKI	PKDSMTLLPC	FYFVELPIVA	SSIVSLYPLE	LTDLPKPAKV	GFQCYDRTLS
70	80	90	100	110	120
MPYVETNEEL	IPLLMLLSLA	FAAPAASIMV	ARGMLYCLQS	RLWGRAGGPA	GAEGSINAGG
130	140	150	160	170	180
CNFNSPLRRT	VRFGVHVFG	LCATALVTDV	IQLATGYHTP	PFLTVCXPNY	TLLGTSCEVN
190	200	210	220	230	240
PYITQDICS	HDTHAILSAR	KTFPSQHEATL	SAFAAVYVSU	SPAPHCPSQA	LLLTRGEPST
250	260	270	280	290	300
TPTPMPQMYF	NSVISDTTKL	LKPILVFAPA	IAAGVCGLTQ	ITQYRSHDVD	VYAGFLIGAG
310	320	330	340	350	360
IAAYLACHAV	GNFOAPPAEK	PAAPAPAKDA	LRALTQRGHD	SVYQONKSVS	TDELGPPGRL
370	380	390	400	410	420
EGAPRPVARE	KTSLGSLKRA	SVDVDLLAPR	SPMAKENMVT	FSHTLPRASA	PSLDDPARRH
430	440	450	460	470	480
MTIHVPLDAS	RSKQLISEWK	QKSLEGRGLG	LPDDASPGHL	RAPAEPMAEE	EEEEDEEEE
490	500	510	520	530	540
EEEEEEDEEG	PAPPSLYPTV	QARPGLGPRV	ILPFRAGPPP	LVIHPEEGAQ	TGAGLSPKSG
550	560	570	580	590	600
AGVRAKWLMM	AEKSGAAVAN	PPRLLOQVAM	SKAPGAPGPK	AAETASSSSA	SSDSSQYRSP
610	620	630	640	650	660
SDRDSASIYT	IDAHAHPHPV	VHLSAGGAPW	EWKAAGGGAK	ABADGGYELG	DLARGFRGGA
670	680	690	700	710	720
KPPGVSPGSS	VSDVDQEEPR	FGAVATYNLA	TGEGLPPLGA	ADGALGPGR	ESTLRREHAGG
730	740	750	760	770	780
LGLAEREAEA	EAEQYFRKMQ	ARRFPD....			

human PRG2

SEQ ID NO: 3

10	20			50	60
MLAMKEKNKT	PKDSMTLLPC	FYFVELPIVA	SSIVSLYFLR	LTDLPKPAKV	GFQCYDRALS
70	80	90	100	110	120
MPYVETNEEL	IPLLMLLSTA	FAAPAASIMV	GEGMVYCLQS	RLNGRQPGGV	EGSINAGGCN
130	140	150	160	170	180
FNSFLRRTVR	FVGVEHVGIC	ATALVTDVIQ	LATGYHTPFF	LTVCKPNYTL	LGTSCSNPY
190	200	210	220	230	240
ITQDICSQHD	THAILSARKT	FPSQHATLSA	FAAVYVSMYF	NAVISDITKL	LKPILVFAPA
250	260	270	280	290	300
IAAGVCGLTQ	ITQYRSHQVD	VYAGFLIGAG	IAAYLACHAV	GNFQAPPARK	VPTPAPAKDA
310	320	330	340	350	360
LRALTQRGHE	SMYQONKSVS	TDELGPPGRL	EGVPRPVARE	KTSLGSLKRA	SVDVDLLAPR
370	380	390	400	410	420
SPMGKEGMVT	FSNTLPRVST	PSLDDPARRH	MTIHVELDAS	RSRQLIGEWK	QKSLEGRGLG
430	440	450	460	470	480
LPDEASPVHL	RAPAEQVAEE	EEEEEEEEER	EEEEEEEEGP	VPPSLYPTVQ	ARPGLGPRVI
490	500	510	520	530	540
LPPRPGFQPL	VHIPEEGVOA	GAGLSPKSSS	SSVRAKWLSV	AEGGGGPVAV	APSQPRVANP
550	560	570	580	590	600
PRLLQVIAMS	KAAGGPKAET	ASSSSASSDS	SQYRSPSDRD	SASIVTIDAN	APHHPVVHLS
610	620	630	640	650	660
AGSTPWENKA	KVVEGEGSYE	LGDLAGFRS	SCKQPGMGPG	SPVSDVDQEE	PRFGAVATVN
670	680	690	700	710	720
LATGEGLPPP	GASEGALGAG	SRESTLRQV	GGLAEREVEA	EAESYYRRMQ	ARRYQD*...

in case

PRG2

SEQ ID NO: 4

10	20			50	60
GGATCCACTA	GTAACGGCTG	CCAGTGTGCT	GGAATTCGCC	CTTGAAGCCA	TTGCAGCAAC
70	80	90	100	110	120
AGCTTGGAGG	AGGGAGCTGG	ACGTCGTCTC	TCGCCAGAAA	AACGGGGAGC	AGGAGCCAGA
130	140	150	160	170	180
CTAAAGGAGG	AAGAGGACTG	GCCCGCTCAG	GGAATAGCTG	GATTGCTGCA	AAAAGggGCG
190	200	210	220	230	240
GggAGAAgGC	GGGGGCGCTG	CATGCAGCgC	GCTGGCTCCA	GCGGTGGCCG	CGGGGAATGT
250	260	270	280	290	300
GACATCAGCG	GCGCCGGGCG	CTTGGGGCTG	GAGGAGGCGG	CTCGCCTCAG	CTGCGCTGTG
310	320	330	340	350	360
CACACCTCGC	CCGGGGGAGG	ACGCAGACCC	GGGCAGGCGG	CAGGGatgtc	ggcgaaggag
370	380	390	400	410	420
aggcdaaagg	gcaaagtgat	caaggacagc	gtcacccctc	tgccctgott	ttatttcgtc
430	440	450	460	470	480
gagttgccta	tattggcata	atcggttggt	agcctctatt	tcctcgaact	cacagatgtc
490	500	510	520	530	540
ttcaaacctg	tgcactctgg	atcttagctgc	tatgacggga	gtcttagcat	gccgtacatt
550	560	570	580	590	600
gaaccaaccc	aggaggcaat	tcatttcctc	atgttgctta	gcttggtctt	tgctggacct
610	620	630	640	650	660
gcaattacga	ttatggtagg	agaaggaatt	ctctactgtt	gcctctccaa	aagaagaaat
670	680	690	700	710	720
ggggtcggac	tagagcccaa	cattaatgct	ggagsgtgc	acttcaattc	cttctcaga
730	740	750	760	770	780
cgagctgtca	gattcggttg	tggtcatgta	tttggattat	gctctacagc	tctcattaca
790	800	810	820	830	840
gatatcatat	agctgtccac	aggatatcaa	gcaccttaet	ttctgactgt	gtgcaaacca
850	860	870	880	890	900
aactatacct	ctctgaatgt	atcttgcaaa	gaaaatttct	acattgtgga	agatatttgc
910	920	930	940	950	960
tcaggatctg	acctcacagt	tatcaadagt	ggcagaaagt	ccttcccttc	tcaacatgca
970	980	990	1000	1010	1020
acccttgotg	cctttgcagc	tggtgatgtt	tcgatgtact	tcaattccac	attaacggat
1030	1040	1050	1060	1070	1080
tcctctaagc	ttctgaaacc	tctcttggtc	ttcacattta	tcattctgtg	aataatctgc
1090	1100	1110	1120	1130	1140
gggctaacac	ggataactca	gtataagaac	caccagtttg	atgtctattg	tggtttttta
1150	1160	1170	1180	1190	1200
ataggaggag	gaattgcact	gtacttggtg	ttgtatgctg	tggggaattt	cctgcocagt
1210	1220	1230	1240	1250	1260
gatgagagta	tgtttcagca	cagagacgcc	ctcaggtctc	tgacagacct	caatcaagat
1270	1280	1290	1300	1310	1320
cccaaccgac	ttttatctgc	taaaaatggt	agcagcagtg	atggaattgc	tcatacagaa
1330	1340	1350	1360	1370	1380
ggcatcctca	accgaaacea	cagagatgct	agctctctga	caaattctca	aagagcaaat
1390	1400	1410	1420	1430	1440
gctgatgtgg	aaatcattac	tccacggagc	cccattggga	aggagaacat	ggttaccttc
1450	1460	1470	1480	1490	1500
agcaatacct	tgcgcgcagc	caatacccca	tctgtagaag	accctgtcag	aagaaatgcg
1510	1520	1530	1540	1550	1560
agcattcatg	cctctatgga	ttccgctcga	tcaaagcagc	tcctcaacca	gtggaagaat
1570	1580	1590	1600	1610	1620
aagaatgaaa	gtcgaaagtt	gtccttgcaa	gttatagagc	ctgagcctgg	gcagtcacca
1630	1640	1650	1660	1670	1680
cccagatcca	tagaaatgag	gtcaagctca	gagccatcga	gggtaggggc	gaatggagac

num 44

PRG 1

START

bp 202

STOP

bp 2490

SEQ ID NO: 1

SEQ ID NO: 5

1690	1700		1730	1740
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1750	1760	1770	1780	1800
aacagcatgc	ctggaggggcc	aagagtgtcc	attcagtcgc	gtccctgggtc
1810	1820	1830	1840	1860
gtgcacatcc	ctgaggagac	tcaggaaaac	ataagcacct	cccccaaaag
1870	1880	1890	1900	1920
cggggccaagt	gggttaaaagc	tgctgaaaag	actgtggcct	gtaacagaag
1930	1940	1950	1960	1980
ccccgaatca	tgcaagtcac	agccatgtcc	aagcagcagg	gtgtccctcca
1990	2000	2010	2020	2040
aagaacactg	aaggcagcac	ggctctcctgc	actggctcca	tcgctataaa
2050	2060	2070	2080	2100
gaccatgagc	ccagtgggat	agtggagggtt	gaggetcacc	cagagaacaa
2110	2120	2130	2140	2160
atcacagatcc	cgtccactga	aggtgaagga	agtggctcct	ggaagtggaa
2170	2180	2190	2200	2220
aagggcagcc	ttcgccaaac	ttacgagctc	aacgatctca	acagggactc
2230	2240	2250	2260	2280
gagtcctctga	aagacagctt	tggttcttga	gatcgcaaga	gaagcaacac
2290	2300	2310	2320	2340
gagcatcacc	accacggaat	taccaccatc	cgcgtcacc	cagtagaggg
2350	2360	2370	2380	2400
ggctcagaga	cgtcttccat	ttcttcttcc	cgcgactcca	ccctgcggag
2410	2420	2430	2440	2460
atcattctaa	tccttgaaag	aagcaacagc	cccgaaaaca	ctagaaatat
2470	2480	2490	2500	2520
ggaacctccc	ccacacgggc	ttataaggat	tgagtgatgt	ccattccatc
2530	2540	2550	2560	2580
ctcgcaaaaag	accatattgt	gattctacct	gtgttctgtc	ccagcgaatt
2590	2600	2610	2620	2640
accaagctag	attgtctacc	atcagcccag	aactctgtaa	cttttcagaa
2650	2660	2670	2680	2700
caaacttgca	gatctcacat	caaggagagg	gaaaagcaca	atgcaagaac
2710	2720	2730	2740	2760
tgatgatatg	aagagttttc	tttaagacctg	tcgtcaaaat	taaaagggtt
2770	2780	2790	2800	2820
agtatcaaaa	gaaagtgggt	ttcttcaaat	gtatactatt	ttacttcttg
2830	2840	2850	2860	2880
ctttggggat	ttttctttat	agtgaagctgt	gggaacccag	aacacacacg
2890	2900	2910	2920	2940
agcagaggec	atgcagtatt	atatattcat	tttcgagaat	ctgcacctac
2950	2960	2970	2980	3000
gggtgggtgt	gattattata	gtacatatac	catgtaaact	ctcaaactct
3010	3020	3030	3040	3060
gaaatagtgg	tgtgcaattc	cttggttaaag	aaatgctact	ttattaagaa
3070	3080	3090	3100	3120
gctttgtgtt	agaataggac	accccgagc	ttctctgtag	tggtctgtgc
3130	3140	3150	3160	3180
aatgaaaagg	tttttgtgag	ttcttcaaaa	attctgtctt	cttcaacatc
3190	3200	3210	3220	3240
tagaaatatt	ttcagtgaag	gggaataaact	agtacttttc	tgcatagtgt
3250	3260	3270	3280	3300
tactttttat	ttaagtatag	gtactgttaa	tgaatctgtt	ttcttagtga
3310	3320	3330	3340	3360
ataattttat	aaatattatt	ttagagaatc	ttttgaaatt	gtcgtgatca

SEQ ID NO

Continuation 1

SEQ ID NO: 5
Continuation 1

3370	3380		3410	3420
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3430	3440	3450	3460	3470
3480				
tttaaaagca	aaaataacaa	ttgacattcc	ttgagcaaaa	tatactgctg
3490	3500	3510	3520	3530
3540				
aacaagaaat	ctgagccaaa	acttgacatt	gtgggttaca	ttgccagaaa
3550	3560	3570	3580	3590
3600				
gtttgcccct	agatgtctac	aactagctgg	cataggttgc	catcttaaea
3610	3620	3630	3640	3650
3660				
aagtccatt	cggttctaca	ttattaactt	tttttttcta	tatcctgatg
3670	3680	3690	3700	3710
3720				
tagagccaca	ctgggttaagt	ttgactogtc	tctaaaaagt	ttttgttaac
3730	3740	3750	3760	3770
3780				
gaggaagaat	ctgaaaaaaa	aatgcatgtt	ggtaagtaaa	agtatctcac
3790	3800	3810	3820	3830
3840				
aagaatgact	ttcttcamaa	tatctgaata	ggtgcagttt	tagtttaaca
3850	3860	3870	3880	3890
3900				
cattggtgct	acctatcctg	aatcaagcct	tgagcctaaa	tcaaagcaaa
3910	3920	3930	3940	3950
3960				
tgataagaag	aagataaaaa	caaaatattt	tggagtgttt	tccaacttaa
3970	3980	3990	4000	4010
4020				
catactcagt	tcttggaaat	tagtattaaa	ccttttttat	gccatttcat
4030	4040	4050	4060	4070
4080				
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4090	4100	4110	4120	4130
4140				
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4150	4160	4170	4180	4190
4200				
caatttccac	atgaaaaagg	tggagcttte	tagaaaaaac	aacctctaag
4210	4220	4230	4240	4250
4260				
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4270	4280	4290	4300	4310
4320				
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4330	4340	4350	4360	4370
4380				
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4390	4400	4410	4420	4430
4440				
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4450	4460	4470	4480	4490
4500				
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4510	4520	4530	4540	4550
4560				
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4570	4580	4590	4600	4610
4620				
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4630	4640	4650	4660	4670
4680				
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4690	4700	4710	4720	4730
4740				
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4750	4760	4770	4780	4790
4800				
atcagactaa	aagggtggat	ctcttcttag	tggtctattt	atcttatttg
4810	4820	4830	4840	4850
4860				
cacttcttcc	tttgttaggc	tgtgctttac	tgataaaaac	aagtattgaa
4870	4880	4890	4900	4910
4920				
taatttatct	tttaaagtta	ATAAAATTAT	GAAAATATAT	ATAGTATATA
4930	4940	4950	4960	4970
4980				
TGTTTAAAAA	AATGTTATGC	AATGTTTTCC	AAACTGATAA	AGTTTGTAAG
4990	5000	5010	5020	5030
5040				
TGTATTTTGT	TAAGtaCAGA	TAAAAGCTAT	TGTGTGAGTA	TATTGTGCTA
				AAATCATAGA

SEQ ID NO: 5

continuation 2

SEQ ID NO: 5

continuation 2

5050

5060

5090

5100

AATAAAGATT AGATTTCITC ATCAAAAAA AAAAAAAA AAA

SEQ ID NO: 5

continuation 3

10	20	30	40	50	60
aggagacgcg	ctttgtgctg	ggcgccggcc	gcgccagcca	cgccctgagg	cgcccgcggc
70	80	90	100	110	120
accatgatct	ccaccaagga	gaagaacaag	atccccgaagg	acagcatgac	gottotgccc
130	140	150	160	170	180
tgcttctact	tcgtggagct	gcccatagtg	gcttcttcca	tcgtatcett	gtacttctg
190	200	210	220	230	240
gagctgaccg	acctcttcaa	gccggccaag	gtgggcttcc	agtgtatga	cgcactctc
250	260	270	280	290	300
tccatgccct	acgtggagac	caacgaggag	ctcatccgc	tgctgatget	gtcagcttg
310	320	330	340	350	360
gccttcgagg	cccctgccgc	ctcgatcatg	gtggccgagg	gcatgttgta	ctgtctgcag
370	380	390	400	410	420
tcccggctgt	ggggcgctgc	cgggggggccc	gccggggcgg	agggcagcat	caacgcgggc
430	440	450	460	470	480
ggctgcaact	tcaactcctt	cctgcggcgt	acgggtgogg	ttgtgggtgt	ccactgtgtc
490	500	510	520	530	540
ggcctgtgtg	ccacagccct	ggtgacggac	gtgatccagc	tggccacggg	ttaccacact
550	560	570	580	590	600
cccttcttcc	tcaccgtctg	caagcccaac	tacactctcc	tgggcacgtc	ctgcgaggtc
610	620	630	640	650	660
aaacctaca	tcacgcagga	catctgctcc	ggccacgaca	tccacgccat	cctgtctgca
670	680	690	700	710	720
cgaagacct	tcccgtccca	gcacgcacag	ctgtcagcct	tcgcccgagg	ctatgtgtcg
730	740	750	760	770	780
gtgagtcagg	caactcactg	cccttcccag	gccctcttgc	tgadccgtgg	ggagccctcc
790	800	810	820	830	840
ctgaccccaa	ccccatgcc	ccagatgtac	ttcaactcgg	tcactctcga	caccaccaag
850	860	870	880	890	900
ctgtgaagc	ccatcctggt	cttcgoottt	gccatcgccg	cggggcgatg	cgggctcagc
910	920	930	940	950	960
cagatcacgc	agtaaccgag	ccaccctgtg	gacgtgtatg	ccggcttccct	catcggggcg
970	980	990	1000	1010	1020
ggcatcgctg	cctacctggc	ctgcccagcg	gtggggcaact	tccaggcccc	acctgcagag
1030	1040	1050	1060	1070	1080
aagcccgagg	cccaggcccc	cgccaaggao	gagctgcggg	ccctgacgca	gcggggccac
1090	1100	1110	1120	1130	1140
gactcgggtt	atcagcagaa	taagtcgggtg	agcacgcagc	agctgggggc	cccaggggcg
1150	1160	1170	1180	1190	1200
ctggagggcg	cgcccggggc	cgtggcccg	gagaagacct	cgtggggcag	cctgaagcgc
1210	1220	1230	1240	1250	1260
gccagcgtgg	acgtggacct	gctggccccc	cgcagcccca	tggccaagga	gaacatgggtg
1270	1280	1290	1300	1310	1320
accttcagcc	acacgctgcc	cagggccagc	gcgcctctgc	tggacgaccc	cgcgcgcggc
1330	1340	1350	1360	1370	1380
cacatgaoca	tccacgtgcc	gctggacgcc	tcgcgctcca	agcagctcat	cagcgagtgg
1390	1400	1410	1420	1430	1440
aagcagaaga	gcctggagggg	ccgcggcctg	gggctgcccc	acgacgccag	ccccgggcac
1450	1460	1470	1480	1490	1500
ctgcgcgcgc	ccgccgaacc	catggcgagg	gaggagggaag	aggaggagga	cgaagaggaa
1510	1520	1530	1540	1550	1560
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1570	1580	1590	1600	1610	1620
gtgcaggcgc	ggccgggggt	ggggcctcgg	gtcatcctcc	cacgcgcgcg	ggggccggcg
1630	1640	1650	1660	1670	1680
cgcctgggtg	acatccggga	ggaggggcgg	cagacggggg	ccggcctgtc	ccccaaaagc

human

PRG 2

START

bp 64

STOP

bp 2304

SEQ ID NO:

SEQ ID NO: 6

1690	1700			1730	1740
ggcgccgggg	cgcgcgdaa	gtggctcatg	atggccgaga	agagcggggc	ggcagtggcc
1750	1760	1770	1780	1790	1800
aaccctccgc	ggctgctgca	ggtcategcc	atgtccaagg	ctccggggcg	gccggggccc
1810	1820	1830	1840	1850	1860
aaggcggccg	agacggcgtc	gtcgtccagc	gceagatccg	actcctcgca	gtaccgggtcg
1870	1880	1890	1900	1910	1920
ccgtcggacc	gcgactccgc	cagcatcggtg	accatcgacg	cgcacgcgcc	gcaccacccc
1930	1940	1950	1960	1970	1980
gtggtgcaac	tgtcggccgg	cggcgcgccc	tgggagtggg	agggcggcgg	cggcgggggc
1990	2000	2010	2020	2030	2040
aaggcggagg	ccgacggcgg	ctacgagctg	ggggacctgg	cgcgcggctt	ccgcggcggg
2050	2060	2070	2080	2090	2100
gccaagcccc	cgggcgtgtc	ccccggctcg	tgggtcagcg	acgtggacca	ggaggagccg
2110	2120	2130	2140	2150	2160
csgttcgggg	ccgtggccac	cgtcaacctg	gccacgggcg	aggggctgcc	cccgtggggc
2170	2180	2190	2200	2210	2220
gcggccgatg	gggcgctggg	cccgggcagc	cgggagtcca	cgetggggcg	ccacgcgggc
2230	2240	2250	2260	2270	2280
ggcctggggc	tggcggagcg	cgaggcgag	gcggaggccg	agggctactt	ccgcaagatg
2290	2300	2310	2320	2330	2340
caggcgcgcc	gcttccccga	ctagcgcgcc	ggggccgggg	ggggcggggg	ggcgggccga
2350	2360	2370	2380	2390	2400
gggcgcgggc	ggcgcg....

SEQ 10

NO. 6

Continuation

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Claims

1. An isolated polypeptide comprising the same or substantially the same amino acid sequence selected from the group consisting of SEQ ID NO:1-4, or a splice variant or a salt thereof.
2. A partial peptide of the protein according to claim 1, or a salt thereof.
3. A DNA which comprises a DNA encoding the protein according to claim 1 and/or 2.
4. A DNA according to claim 3 selected from the group consisting of SEQ ID NO:5-6.
5. A recombinant vector which comprises the DNA according to claim 3.
6. A transformant transformed with the recombinant vector according to claim 5.
7. A method of producing the protein or its salt according to claim 1, which comprises culturing the transformant according to claim 6, and producing and accumulating the protein according to claim 1.
8. An antibody to the protein according to claim 1, the partial peptide according to claim 2, or a salt thereof.
9. A method of determining a ligand to the protein or its salt according to claim 1, which comprises using the protein according to claim 1 or the partial peptide according to claim 2, or a salt thereof.
10. A pharmaceutical composition comprising a polypeptide and/or DNA according to one of the claims 1-5.

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13. Sep. 2002

Abstract

Outgrowth of axons in the central nervous system is governed by specific molecular cues. Molecules detected so far act as ligands that bind to specific receptors. Here, we report on a novel membrane-associated lipid phosphate phosphatase we named plasticity-related gene-1 (PRG-1), which facilitates axonal outgrowth during development and regenerative sprouting. PRG-1 is specifically expressed in neurons and is located in the membranes of outgrowing axons. There, it acts as an ecto-enzyme and attenuates phospholipid-induced axon collapse in neurons and outgrowth in the hippocampus. Thus, we unraveled here a novel mechanism by which axons are able to control phospholipid-mediated signaling and overcome the growth-inhibiting phospholipid-rich environment of the extracellular space.

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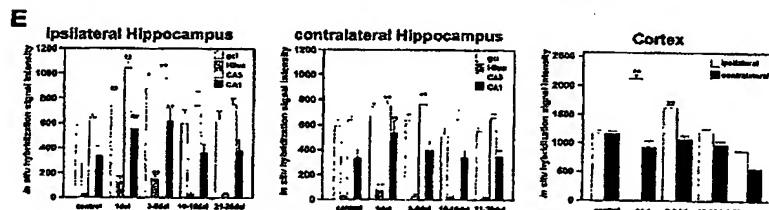
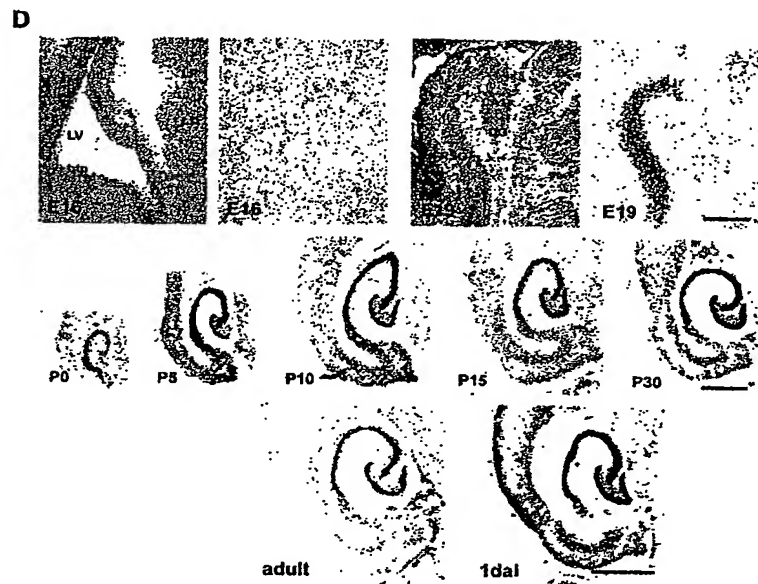
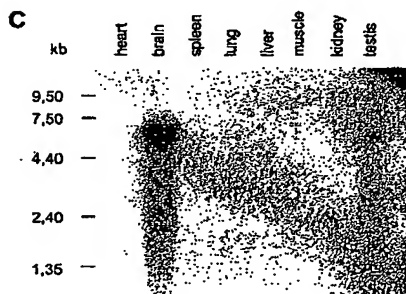
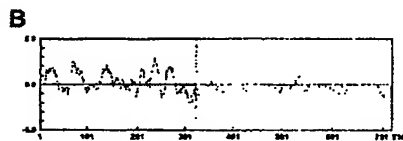
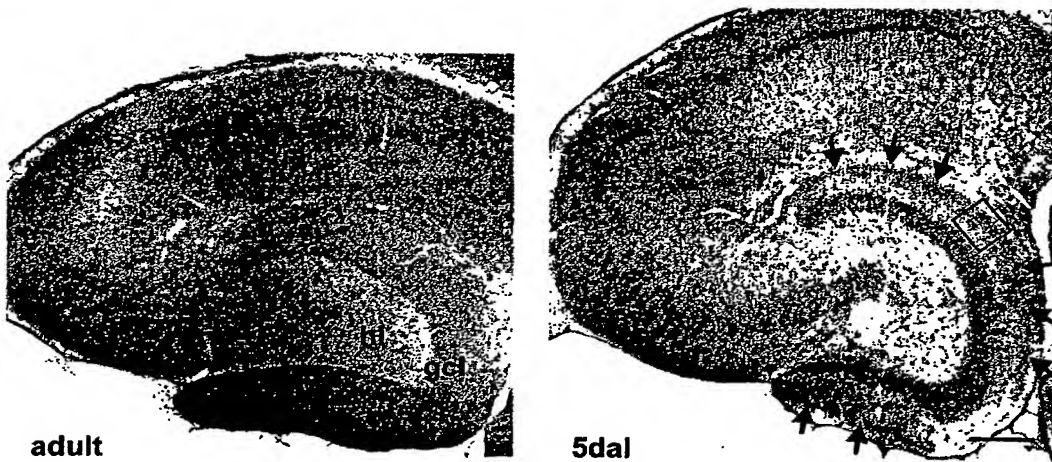


Fig. 2

A



B



C

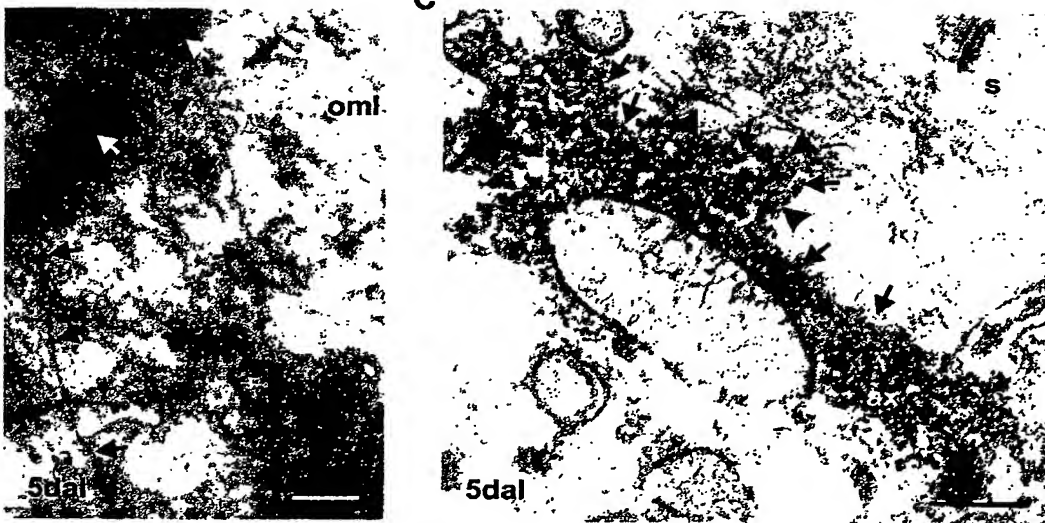
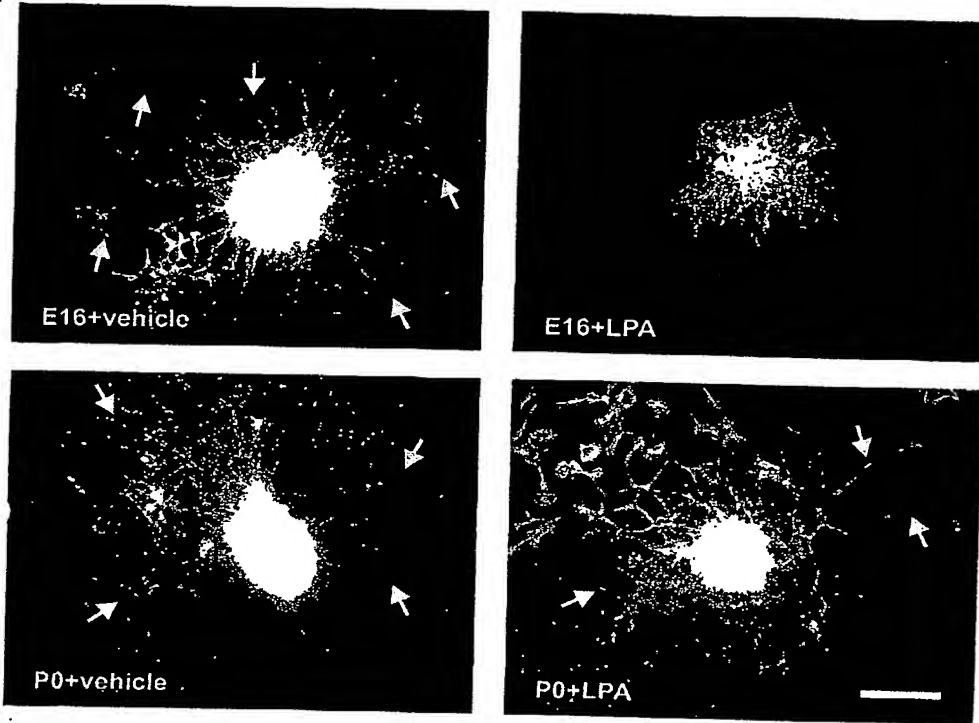


Fig. 3

A



B

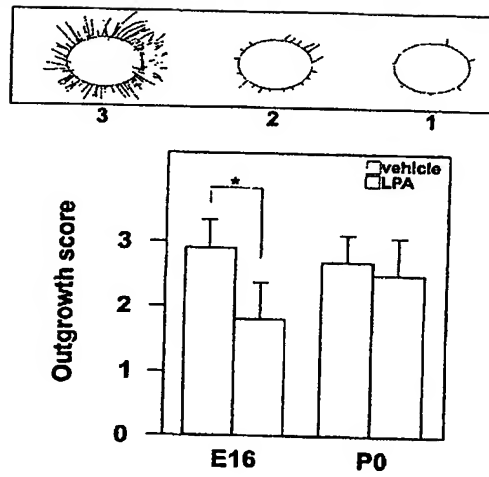


Fig. 4

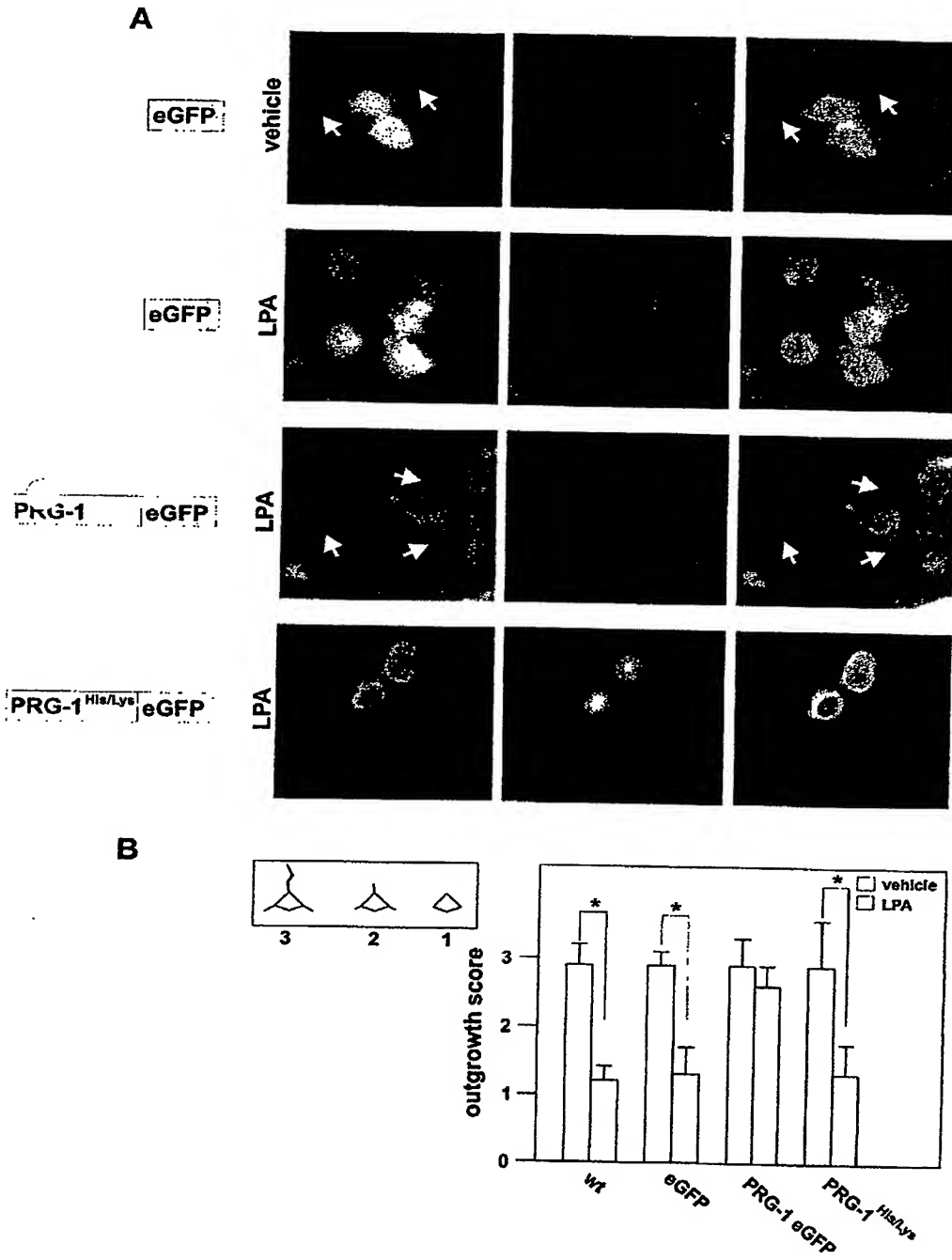
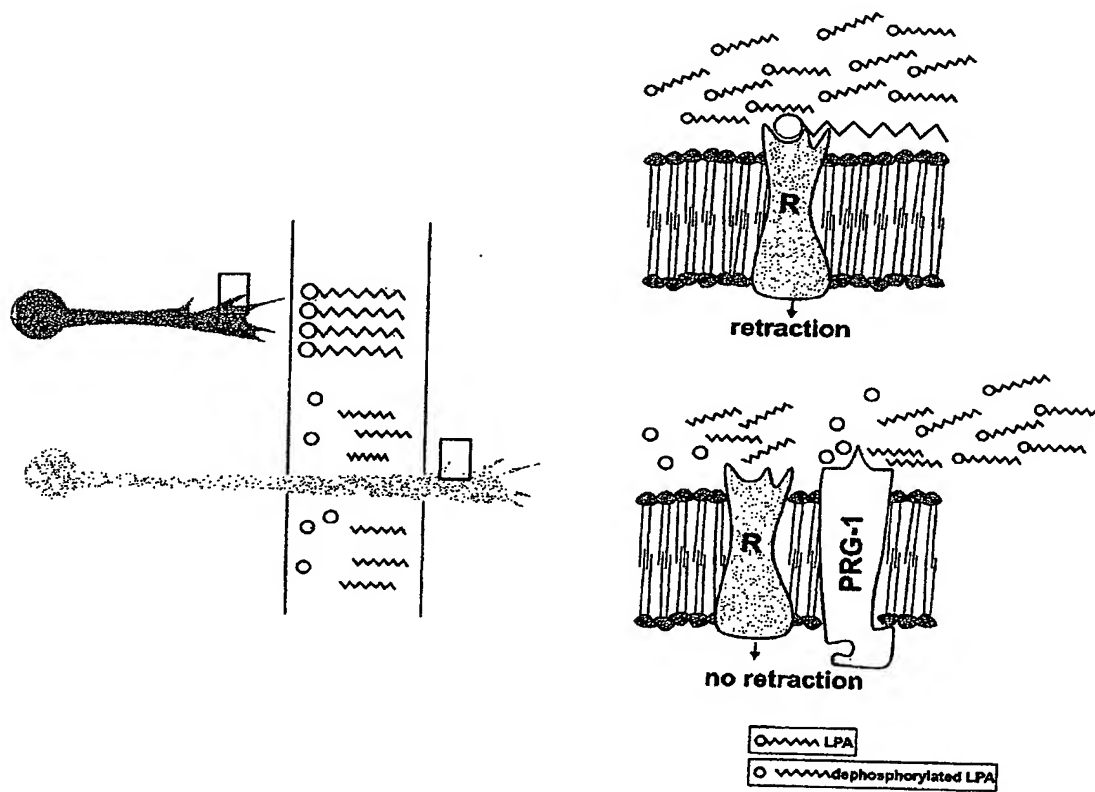


Fig. 5



SCHEMATIC DIAGRAM

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